Remarks/Argument

Claims 1-20 are pending in the application. Claims 3, 4, 6, 10, 12, 13, 15 and 16 have been cancelled. New claims 21 and 22 have been added.

Claims 1, 2, 5, 7-9, 11, 14 and 17-20 have been amended. Support for amended claims 1 and 14 can be found on pg. 8, lns. 10-17 and pg. 13, lns. 16-18 of the specification, and in original claims 3 and 4. Support for amended claims 7 and 18 is found on pg. 5, lns. 17-18 of the specification and in original claim 3. Support for amended claims 9 and 20 is found on pg. 10, lns. 12-13 of the specification. Support for amended claim 11 is found on pg. 10, lns. 12-14 of the specification.

Support for new claim 21 is found on pg. 10, lns. 14-16 of the specification.

Support for new claim 22 is found on pg. 8, ln. 30 to pg. 9, ln. 11 of the specification.

After entry of this Response, claims 1, 2, 5, 7-9, 11, 14 and 17-22 will be pending in the application. Based on the above changes and the following remarks, Applicant respectfully requests reconsideration of the claims and withdrawal of the pending rejections.

Response to Section 112, 2nd paragraph and Section 101 rejections

Claims 1, 3, 7 and 8 are rejected under 35 U.S.C. §112, 2nd paragraph as being indefinite for allegedly failing to recite any positive process steps and allegedly failing "to particularly point out and distinctly claim the subject matter which the applicant regards as the invention." These claims are also rejected under 35 U.S.C. §101 as allegedly being improper process claims. Claim 3 has been cancelled, and this rejection is moot as to that claim.

Claim 1 as amended is directed to a process for disrupting target gene expression with RNA interference ("RNAi"). The sentence bridging pgs. 2-3 of the present specification states that RNAi is triggered by introducing double stranded RNA ("dsRNA") into cells, where endogenous cellular machinery degrades the corresponding mRNA from the target gene in a sequence-specific matter. Claim 1 has therefore been amended to recite that RNAi is initiated by the positive process step of exposing cells to double stranded RNA which is homologous to the target gene. With this amendment,

claim 1 and its dependent claims 7 and 8 are believed to comply with 35 U.S.C. §112, 2nd paragraph and §101, and Applicant respectfully requests that these rejections be withdrawn.

Claims 4-6, 9-11 and 14-20 are rejected as indefinite because each of these claims defines "further steps" to the process recited in claim 1. Claims 4, 6, 10, 15 and 16 have been cancelled, and this rejection is moot as to those claims.

According to the Examiner, because claim 1 has no positive process steps, the recitation of further process steps in dependent claims 5, 9, 11, 14 and 17-20 renders these claims unclear. As discussed above, claim 1 has been amended to include the positive process step of exposing cells to a double stranded RNA in order to initiate RNA interference. Thus, the definition of further process steps in claims 5, 9, 11, 14 and 17-20 is clear and definite, and Applicant respectfully requests that the indefiniteness rejection of these claims be withdrawn.

Claim 4 is rejected as indefinite for reciting the phrase "the target gene encoding the disrupted expression." Claim 4 has been cancelled, and this rejection is now moot.

Claims 5, 6 and 9-11 are rejected as indefinite for depending from claim 4. Claims 6 and 10 have been cancelled, and this rejection is moot as to those claims. Claims 5, 9 and 11 have been amended to depend (directly or indirectly) from claim 1. Applicant respectfully requests that the indefiniteness rejection of claims 5, 9 and 11 be withdrawn.

Claim 5 is rejected as indefinite because it is allegedly unclear what further process step is defined. Claim 5 now depends from claim 1, which recites a process for disrupting target gene expression with RNAi by exposing cells to double stranded RNA which is homologous to the target gene. Claim 5 has also been amended to specify that the function of the target gene is disrupted by RNAi. However, the disruption of target gene function is now presented in claim 5 as an effect, and not as a further process step. Applicant respectfully requests that the indefiniteness rejection of claim 5 be withdrawn.

Claims 6, 9, 10 and 11 are rejected as indefinite because it is allegedly unclear how the method steps recited in each of these claims are related to the method recited in the respective parent claims. Claims 6 and 10 have been cancelled, and this rejection is moot as to those claims.

Claims 9 and 11 (which had depended on claim 6) have been amended to depend on claim 1, now specify that the dsRNA to which the cells are exposed is prepared as a pharmaceutical composition. Applicant believes that claims 9 and 11 are clear and definite, and respectfully requests that the indefiniteness rejection of these claims be withdrawn.

Claim 11 is rejected as indefinite for reciting the phrase "the RNA-based drugs," which allegedly has no antecedent basis. This phrase has been deleted from claim 11, and Applicant respectfully requests that the indefiniteness rejection of this claim be withdrawn.

Claims 1-11 and 14-20 are rejected as indefinite for reciting the phrase "disrupting target cell expression at the mRNA level" (emphasis added). Claims 3, 4, 6, 10, 12, 13, 15 and 16 have been cancelled, and the rejection is moot as to these claims. Claims 1 has been amended to recite "disrupting target gene expression at the mRNA level," and claim 14 has been amended to recite "disruption of target gene expression at the mRNA level". These amendments to claims 1 and 14 to reflect that it is the mRNA transcribed from the target gene which is degraded by the claimed RNAi method. Applicant respectfully requests that the indefiniteness rejection of claims 1, 2, 5, 7-9, 11, 14 and 17-20 be withdrawn.

Claims 14-20 are rejected as indefinite for reciting the phrase "using RNA interference." Claims 15 and 16 have been cancelled, and the rejection is moot as to these claims. According to the Examiner, it is not clear whether this phrase indicates that method steps beyond the administration of dsRNA are necessary to mediate RNAi. The phrase "using RNA interference" has been deleted from claim 14, in order to more particularly point out that no further step beyond the administration of dsRNA is necessary to cause disruption of target gene expression by RNAi. Applicant respectfully requests that the indefiniteness rejection of claims 14 and 17-20 be withdrawn.

Response to Section 112, 1st paragraph rejections

Claims 14-20 are rejected under 35 U.S.C. 112, 1st paragraph as allegedly lacking enablement for treating an RNA-based disease or disorder in any mammal, where a therapeutic outcome is achieved. Claims 15 and 16 have been cancelled without

prejudice, and the rejection is therefore moot as to these claims. The rejection will be discussed as to claims 14 and its dependent claims 17-20.

According to the Examiner, the field of RNAi was highly unpredictable at the time the present application was filed. Therefore, undue experimentation would allegedly be needed to practice the method recited in claims 14 and 17-20. Applicant respectfully disagrees.

Claim 14 has been amended to recite that a human subject is treated for an RNA-based disorder or disease. As discussed below, the present specification is enabling for methods of treating RNA-based diseases or disorders in human subjects by silencing abnormal gene expression through targeting its encoded RNA.

To be enabling, a specification must teach one skilled in the art how to make and use the claimed invention without undue experimentation. Here, the specification clearly and extensively teaches how to make dsRNA (or pharmaceutical compositions of the dsRNA) for administration to a human subject. See, *e.g.*, pg. 9, ln. 12 to pg. 10, ln. 11 and on pg. 11, ln 1 to pg. 12, ln. 20. Page 10, lns. 13-16 teach that the dsRNA can be used to treat human RNA-based diseases or disorders, in particular, cancer. The administration of the dsRNA or pharmaceutical compositions thereof is discussed on pg. 10, lns. 19-24 and on pg. 11, lns. 1-6. In particular, the specification states at pg. 11, lns. 1-2 of the present specification that:

> "[i]t will be understood by those skilled in the art that any mode of administration vehicle or carrier conventionally employed and which is inert with respect to the active agent may be utilized for preparing and administering the pharmaceutical compositions of the invention."

Examples of such conventionally employed modes of administration can be found in the art. For example, Jen et al. (2000), *Stem Cells* 18: 307-319 (cited by the Examiner, and co-authored by the Applicant, Alan Gewirtz) details numerous techniques for delivering nucleic acids to target cells *in vivo*, including delivery of naked nucleic acid and delivery with agents such as cationic liposomes, cationic porphyrins, polyethylenimine, fusogenic peptides and artificial virosomes. Although (as the Examiner notes) Jen et al. state that "efficient delivery for *in vivo* animal studies remains questionable," Greene et al. (2000), *J. Am. Coll. Surg.* 191: 93-105 (also cited by the Examiner) state on pg. 97 that "[m]any of the limitations to antisense studies outlined

earlier have been relatively easily dealt with," and that "the *in vivo* delivery of (antisense oligonucleotides) appears to be more efficient that that which occurs *in vitro*."

Applicant points out that the mechanisms by which single-stranded antisense oligodeoxynucleotides and dsRNA disrupt gene expression differ greatly. Single-stranded antisense oligodeoxynucleotides bind directly to a target mRNA in a 1:1 ratio, and disrupt gene expression by blocking translation of the target mRNA, or by activating RNase H which degrades the target mRNA involved in the duplex. This process is entirely dependent on the appropriate targeting of the single-stranded antisense oligodeoxynucleotides such that its sequence is complementary to a single stranded region of the target mRNA. If the single-stranded antisense oligodeoxynucleotide is complementary to a region involved with intramolecular duplex formation, hybridization cannot take place, and neither of the two possibilities just described (block in translation or destruction of mRNA) can take place. In contrast, dsRNA initiate RNAi by undergoing cleavage into appropriately sized double stranded fragments (21-22 nts) which are then incorporated into an RNA-induced silencing complex or "RISC." The RISC has two important attributes: (1) It can "melt" (denature) mRNA structure by its helicase activity, thereby making sequence selection of the antisense strand less stringent, and (2) it does not have to recruit RNaseH since it also possesses RNase activity. These properties render RNAi-induced gene silencing more reliable and efficient than a randomly chosen single-stranded antisense oligodeoxynucleotides employed for a similar purpose. Accordingly, the unique manner in which RNAi causes disruption of gene expression precludes a direct comparison with the antisense mechanism. Applicant wishes to emphasize that the method of this invention, dsRNA-induced RNAi, is not antisense technology. However, as both antisense and RNAi methods require the delivery of nucleic acids to target cells, the teachings of the antisense art with regard to nucleic acid delivery have relevance to the claimed RNAi methods.

For example, Greene et al. provide numerous examples of *in vivo* studies in which antisense oligonucleotides were successfully delivered to human patients. See, *e.g.*, pgs. 99-102 of Greene et al., which detail the successful delivery of antisense oligonucleotides to target cells in humans by: intravenous infusion (to treat lymphoma, steroid-dependent

Crohn's disease and HIV); subcutaneous administration (to treat non-Hodgkin's lymphoma and prostate cancer); and intravitreal injection (to treat AIDS-related cytomegalovirus infection). Techniques for the successful delivery of nucleic acid-based therapeutic agents are thus well-known in the art, and one skilled in the art would understand that such delivery techniques are suitable for delivery of RNAi therapeutic agents.

Because delivery techniques for nucleic acid-based therapeutic agents are well-known in the art, such techniques need not be disclosed in detail in the present application. See MPEP 2164.05(a), which states that "[t]he specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public."

The Examiner contends that undue experimentation is needed to practice the presently claimed RNAi methods. Applicant respectfully disagrees. In the "unpredictable" arts, such as certain aspects of chemistry and biotechnology, some experimentation may be required to identify compounds and methods which fall within the scope of the claims. However, as long as the experimentation does not "require ingenuity beyond that to be expected of one of ordinary skill in the art," the experimentation is not undue. In re Angstadt, 190 USPQ 214, 218 (CCPA 1976), citing Fields v. Conover, 170 USPQ 276, 279 (1971).

In Angstadt, the patent in suit disclosed a complex catalyst comprising a hexaalkylphosphoramide and a transition metal salt to catalyze the oxidation of secondary or tertiary alkylaromatic hydrocarbons to form hydroperoxides. An exemplary list of transition metal salt catalysts was provided, as well as a description of how to test a putative complex catalyst. The patent also described a finite result (the formation of hydroperoxides) as the signal that a putative complex catalyst fell within the scope of the claims. The CCPA held that:

> "If one skilled in this art wished to make and use a transition metal salt other than those disclosed in appellants' 40 runs, he would merely read appellants' specification for directions how to make and use the catalyst complex to oxidize the alkylaromatic hydrocarbons, and could then determine whether hydroperoxides are, in fact, formed. The process discovered by appellants is not complicated, and there is no indication that

> special equipment or unusual reaction conditions must be provided when practicing the invention."

In re Angstadt at 218. Thus, if the disclosed process is not complicated to those of ordinary skill in the art, and no unusual or reaction conditions or special equipment is required, then simply following a patent disclosure to determine if a compound falls within the scope of the claims does not constitute undue experimentation.

Here, Applicant has shown for the first time that RNAi can be reliably and predictably practiced in human cells. The claims of the instant application, as amended, are therefore directed to a method of treating a human subject by administering a dsRNA preparation wherein the dsRNA is homologous to a target gene. The dsRNA administered to the human subjects initiates RNAi, which disrupts target gene expression at the mRNA level. Once presented with the teachings of the instant application, Applicant submits that one skilled in the art could practice the claimed RNAi method without undue experimentation.

For example, one skilled in the art can readily identify the sequence of a target gene, and obtain a dsRNA which is homologous to that gene. It is important to note that the dsRNA can contain some or all of the cDNA sequence for a given target gene. Because the dsRNA is processed by endogenous cellular machinery into multiple small interfering RNA (siRNA) "guide sequences" which target different portions of the target mRNA (see, *e.g.*, pg. 8, ln. 30 to pg. 9, ln. 11 of the present specification), it is not necessary to identify and choose individual target sequences, from which individual siRNA molecules must then be constructed.

The ability of a dsRNA to induce gene-specific RNAi can be readily tested by exposing a human cell (either *in vivo* or *in vitro*) to the dsRNA and measuring the level of mRNA or protein produced by the target gene. Exemplary techniques for obtaining dsRNA, exposing cultured human cells to the dsRNA, and measuring the level of mRNA produced from the target gene are given in the present specification on pgs. 12-14. The techniques outlined on pgs. 12-14 of the specification are standard molecular biology and cell culture techniques which are well-known to those skilled in the art, and no unusual reaction conditions or special equipment are required to perform them. One skilled in the art can, therefore, by simply following the teachings of the present specification (in

particular pgs. 12-14), obtain dsRNA and use it to induce gene-specific RNAi in human cells as recited in the present claims. The amount of experimentation needed to carry out the claimed RNAi method is therefore not undue.

The Examiner relies on certain examples from the recently-published scientific literature to support her contention that therapeutic RNAi is unpredictable and that, therefore, the presently claimed RNAi methods are not enabled. For example, pg. 8 of the office action states that Oates et al. (2000), *Dev. Biol.* 224: 20-28 and Wianny et al. (2000), *Nature Cell Biol.* 2: 70-75 show RNAi in mammalian cells to be unpredictable or transient.

Oates et al. studied RNAi in zebrafish embryos, and concluded that "double-stranded RNA is not a practical technique for investigating zygotic gene function during early zebrafish development" (Oates et al. abstract). The statement in Wianny et al. regarding the unpredictability of RNAi also relates to zebrafish embryo studies; see pg. 74, 1st paragraph of the 1st column. Zebrafish are, of course, not humans. The zebrafish embryo RNAi experiments discussed in Oates et al. and Wianny et al., which purport to show the "unpredictability" of RNAi, are therefore not relevant to or predictive of human systems.

The Examiner also states on pg. 8 of the office action that the presence of dsRNA-specific adenosine deaminase (dsRAD) activity in *Xenopus*, the African clawed frog, "would predict that dsRNA methods would not be effective (see for example Wianny et al., pg. 74)." As with the zebrafish embryo RNAi studies discussed above, the conclusions drawn from studies on frog cells have little bearing on human cells. In any case, according to Wianny et al., "dsRNA modified by (dsRAD in *Xenopus*) might actually mediate RNAi." See Wianny et al., pg. 74, 1st paragraph of 1st column.

The Examiner further notes that Wianny et al. show only a transient knockdown of gene expression in the mouse with RNAi. According to the Examiner, such transient gene knockdown means that RNAi would be ineffective as a therapeutic tool. However, Wianny et al. showed that RNAi could be induced in mammalian cells for a sufficient time to observe a phenotypic effect.

Wianny et al. demonstrated that dsRNA injected into mouse oocytes inhibited gene expression for more than six rounds of cell division. In some instances, this

corresponded to a 100-fold increase in cell number. See Wianny et al., pg. 74, 2nd paragraph of 1st column. According to Wianny et al., "the effects of RNAi persist for sufficient time to phenocopy the loss of gene function" (pg. 74, 2nd paragraph of 1st column). In other words, a phenotypic change in the cells could be observed, even though the inhibition of gene expression by RNAi was not permanent.

Here, Applicant has demonstrated for the first time in the instant application that RNAi can be induced in human cells. One could not predict from Wianny et al.'s mouse experiments that human RNAi could be reliably performed; however, once demonstrated by the instant invention, one skilled in the art would not and should not dismiss the claimed RNAi method as a therapeutic tool for humans because of Wianny et al.

The transient nature of RNAi, as observed for example in Wianny et al., is not necessarily a detriment, and in fact can be a desirable trait, in a therapeutic agent. For example, most therapeutic agents are designed to be cleared from the body after a certain time, so that their pharmacologic effect is not permanent. Ideally, a drug is cleared or is inactivated after the condition which it was designed to treat is relieved. In this respect, RNAi therapeutics approach this ideal, because these agents stimulate the RISC complex, which operates only as long as the target gene is transcribing RNA. After RNA transcription from the target gene stops, and all RNA transcripts have been destroyed by the RISC, the RNAi mechanism is "switched off." Another application of the RNAi therapeutic agent is then required to activate the RNAi mechanism again.

In any case, many genes of therapeutic interest are only active at discrete times during the cell cycle or during tissue growth or development. Therefore, a therapeutic effect can be achieved by inducing RNAi of such genes at the critical time, where the absence of the gene product would produce the desired phenotypic effect. The transient nature of RNAi, as shown in Wianny et al. and as discussed by the Examiner, would therefore not cause one of ordinary skill in the art to doubt the therapeutic effectiveness of agents which induce RNAi.

The Examiner also contends that the PKR response, which is often present in mammalian cells, "would preclude the use of double stranded RNA *in vivo*" (see pg. 8 of the office action). The PKR response is an intracellular defense mechanism designed to inhibit viral infection, which operates by shutting down translation in a cell in the

presence of long dsRNA. This shutting-down of translation ultimately leads to the death of the cell by apoptosis. However, the present specification demonstrates that specific inhibition of gene expression by RNAi of this invention can be induced in human cells by long dsRNA, as recited in the present claims.

For example, the experiments described on pg. 12, ln. 23 to pg. 14, ln. 6 of the present specification show that KitR expression was significantly reduced ($p<0.01$) in human cancer cells treated with 150 or 250 μg/ml c-Kit dsRNA. However, no reduction in KitR expression was seen upon treatment of the cells with control green fluorescent protein (GFP) dsRNA. If the inhibition of KitR expression upon administration of c-Kit dsRNA was due to a general inhibition of translation via the PKR response, then a similar reduction in KitR expression should have been induced by the GFP dsRNA. However, since KitR expression was reduced only upon administration of c-Kit dsRNA, the inhibition of KitR expression was due to gene-specific RNAi of this invention. Thus, the present specification, in describing and enabling the claimed invention, demonstrates the feasibility of inducing gene-specific RNAi in human cells with dsRNA without apparent interference from the PKR response.

In any case, the presence of a PKR response in a given cell does not necessarily render RNAi ineffective. For example, the Examiner contends on pg. 8 of the office action that the use of shorter dsRNA molecules overcomes the PKR response and results in more reliable and predictable inhibition of gene expression. However, it is not the case that shorter dsRNA molecules avoid the PKR response. See, *e.g.*, Sledz et al. (2003), *Nature Cell Biol.* 5: 834-839 (copy enclosed), who showed that both siRNA and longer dsRNA evoke the PKR response. However, as Sledz et al. state on pg. 837, "the IFN (*i.e.*, PKR) system neither facilitates RNAi mediated by 21-bp siRNAs, nor prevents RNAi mediated by 500-bp IR RNA." This conclusion comports with the data presented in the instant application.

The data presented in the specification also show that the RNAi-induced inhibition of target gene expression in human cells produces a therapeutic effect, as recited in the present claims. See, *e.g.*, pg. 14, lns. 17-25 of the present specification, which describes the inactivation of Lyn receptor tyrosine kinase upon RNAi inhibition of KitR expression induced by the c-Kit dsRNA.

Receptor tyrosine kinases (RTKs), such as Lyn kinase, are typically involved in transmitting extracellular signals for controlling the growth and differentiation of cells. RTKs are thus well-known and attractive therapeutic targets for anticancer agents. See, *e.g.*, Bennasroune et al. (2004), *Crit. Rev. Oncol.* 50: 23-28 (copy enclosed). The ability of Lyn kinase to autophosphorylate in the presence of stem cell stimulating factor (SCF) is a known downstream effect of KitR engagement (see pg. 14, ln. 18 of the present specification). One skilled in the art would therefore view Lyn kinase as a viable target for an anticancer therapeutic agent.

As discussed above, the activation of Lyn kinase through autophosphorylation was reduced in human cancer cells treated with c-Kit dsRNA, and this reduction in Lyn kinase activation correlated with the RNAi-induced reduction in KitR expression. The RNAi-induced inhibition of KitR expression, with the concomitant reduction in the ability of Lyn kinase to autophosphorylate, therefore demonstrates that the claimed RNAi method has a therapeutic effect in human cells.

The Examiner has indicated that the working examples in the present specification, which show inhibition of KitR expression by RNAi in two human cancer cell lines, are insufficient to enable the full scope of the claimed methods. However, there is no requirement that the working examples, of themselves, fully support a claimed invention. Rather, the specification *taken as a whole* must be enabling. In re Barr, 170 USPQ 330 (CCPA 1971).

Here, the working examples show that RNAi can be induced in CHP 100 neuroepithelioma (melanoma) and HL-60 leukemia cells, which represent two different human cancer cell lines from different tissues and developmental origins. Because the instant application shows that RNAi can be induced in such widely divergent human cancer cell lines, one skilled in the art would reasonably believe that RNAi could be induced in any human cell. Thus, the present specification (including the working examples) contains ample direction on how to practice the full breadth of the claimed RNAi therapeutic method.

On pgs. 8-11 of the office action, the Examiner has cited and discussed several references published after the filing date of the present application to support her contention that RNAi therapeutic methods are unpredictable, stating on pg. 9 that:

> RNA interference methods for therapeutic methods encounter the same problems long recognized in other nucleic acid based therapies, particularly with regard to the inability to specifically deliver an effective concentration of a nucleic acid to a target cell, such that the target gene is inhibited to a degree necessary to result in a therapeutic effect.

The Examiner analogizes the problems encountered in antisense therapies to those which are (ostensibly) expected to be encountered in RNAi therapeutic methods, by virtue of a statement from Caplen NJ (2003), *Expert Opin. Biol. Ther.* 3: 575-586, who indicates that antisense and RNAi therapies have similar impediments. However, although antisense and RNAi therapies do both involve the administration of nucleic acids, the two techniques vary so significantly in their mechanism of action that none of the impediments to antisense technology identified by the Examiner applies to the claimed RNAi method.

For example, antisense oligonucleotides often cannot access their intended target site on an mRNA molecule, due to secondary structure in the mRNA. In contrast, the presently claimed methods involve the administration of dsRNA to a subject, which dsRNA is homologous to a target gene. This dsRNA is processed by endogenous cellular nucleases into 21-23 nucleotide siRNA "guide sequences" targeted to different parts of the target mRNA. These siRNA "guide sequences" are incorporated into RISC, which then degrades the target mRNA in a catalytic manner. Thus, the multiple siRNA "guide sequences" produced from a given dsRNA ensure multiple targeting of the target mRNA. See, *e.g.*, pg. 8, ln. 30 to pg. 9, ln. 11 of the present specification. Also, the RISC contains a helicase which "melts" any secondary structure which may be present in the target RNA molecules. The problem of target site accessibility encountered in antisense techniques is, therefore, not present in the claimed RNAi method.

The articles cited by the Examiner also discuss the problem of "non-specific" or "non-antisense" effects of antisense oligonucleotides. These "non-specific" or "non-antisense" effects refer to the difficulty that some researchers have had in distinguishing true antisense inhibition of gene expression (*e.g.*, by RNase H activity or by blocking translation) from some other mechanism induced by the antisense oligonucleotide. However, as discussed on pgs. 4 and 8 of the present specification, it is accepted by those skilled in the art that dsRNA is processed into siRNA "guide sequences," which induce

the RISC and effect gene-specific RNAi. Other than the possible PKR response, which may mask the gene-specific RNAi, a dsRNA does not appear to induce any non-specific effects with regard to inhibition of gene expression. As discussed above, the present specification shows that RNAi can be induced in human cells without apparent interference from the PKR response. Therefore, one skilled in the art would not consider the induction of "non-specific" or "non-antisense" effects by antisense oligonucleotides to be relevant to the claimed RNAi method.

The articles cited by the Examiner also discuss the difficulty of delivering a sufficient concentration of antisense oligonucleotides to a target cell, so that the target mRNA is degraded. As discussed above, various techniques for the effective delivery of nucleic acids to target cells are well-known in the art (see, *e.g.*, Greene et al., discussed *supra* and cited by the Examiner). However, even with effective delivery to target cells, antisense oligonucleotides often do not sufficiently inhibit target gene expression. This is because antisense oligonucleotides bind to their target mRNA molecules on a one-to-one basis. If the number of mRNA molecules in a cell is greater than the number of antisense oligonucleotides delivered to that cell, then gene expression is only partially inhibited.

In contrast, the RNAi-induced destruction of mRNA induced with the presently claimed method is sub-stoichiometric; *i.e.*, one dsRNA molecule can destroy hundreds or even thousands of mRNA molecules by induction of the RISC. The RISC operates to destroy virtually all mRNA present which is homologous to dsRNA, as directed by the siRNA "guide sequences." Thus, only a few molecules of dsRNA need be delivered to a target cell in order to significantly inhibit gene expression through RNAi. The problem of delivering sufficient amounts of material to a cell in order to produce the desired inhibition of gene expression encountered in antisense therapies is therefore not present in the claimed RNAi methods.

Moreover, in antisense technology, recruitment of RNase H from the nucleus to cleave the RNA:DNA duplex can be a limiting factor. However, the nuclease which cleaves the target RNA in RNAi is already in the RISC complex; thus, there is no need to recruit a nuclease from the nucleus to cleave the target molecule.

The Examiner also states on pg. 11 of the office action that "[d]ue to differences in the physiological conditions of a cell *in vitro* versus *in vivo*, the uptake and biological

activity observed *in vitro* would not predictably translate to *in vivo* results. The initial burden is on the Examiner to give reasons as to why an *in vitro* model is not correlative of *in vivo* results. M.P.E.P. 2164.02. However, no specific reasoning has been given refuting that one skilled in the art would accept the *in vitro* human cancer cell data shown in the present specification as reasonably predictive of a therapeutic effect *in vivo*.

Human cancer cell lines have long been used as models of *in vivo* disease, and one skilled in the art would accept that there is a reasonable correlation between the disclosed *in vitro* utility, based on human cancer cell experiments, and the claimed *in vivo* activity of the dsRNA. A rigorous correlation between the *in vitro* model and the *in vivo* activity as herein claimed is therefore not necessary for enablement of the claimed RNAi methods, M.P.E.P. 2164.02, nor is it possible prior to FDA approval of a dsRNA-based therapeutic.

Thus, the present specification gives ample guidance on how to make the claimed dsRNA, and how to use the dsRNA to effect RNAi of a target gene in a human cell. The data presented in the present specification show that RNAi-specific effects can be produced without unduly triggering the PKR response, and that one skilled in the art would accept that a therapeutic effect can be achieved *in vivo*. The unique nature of RNAi, including the ability of dsRNA to form multiple siRNA "guide sequences" and to induce the destruction of hundreds or thousands of target mRNA molecules, avoids the problems encountered with antisense technology. Moreover, one skilled in the art can follow the teachings of the specification and practice the claimed RNAi methods without undue experimentation. Claims 14 and 17-20 are thus enabled for treating an RNA-based disorder or disease in a human subject, by administering dsRNA to induce gene-specific RNAi. Applicant, therefore, respectfully requests that the rejection of claims 14 and 17-20 under 35 U.S.C. 112, 1st paragraph be withdrawn.

Conclusion

Based on the foregoing, all pending claims are believed in condition for allowance. An early and favorable action toward that end is earnestly solicited.

Respectfully submitted,

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